IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
WOLFMAN *et al.*	)	Group Art Unit: 1649
	)	
Application No.: 10/689,677	)	Examiner: Aditi Dutt
	)	
Filed: October 22, 2003	)	Confirmation No.: 2405
	)	
For: ACTRIIB Fusion Polypeptides and Uses Therefor	)	
	)	
	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION OF DR. PAUL YAWORSKY UNDER 37 C.F.R. § 1.132

I, Paul Yaworsky, declare:

1. I am an Associate Director, Women's Health and Musculoskeletal Biology, at Wyeth and have been employed at Wyeth (and its predecessor company, Genetics Institute) in various scientific capacities since March 1998. I received my Ph.D. in Molecular Neuroscience from the Mayo Clinic in 1997, and my B.A. in Molecular Genetics from the University of Toronto in 1989.

2. I have reviewed and understand U. S. Patent Application No.10/689,677 (the '677 application), in the names of inventors Neil M. Wolfman and Mary Bouxsein,

including the claims set forth in the response accompanying this declaration. The claims of the '677 application relate to methods for administering ActRIIB-Fc fusion polypeptides for the treatment of muscular and neuromuscular diseases and/or disorders.

3. As part of my work at Wyeth, my research group has evaluated the ability of ActRIIB-Fc fusion polypeptides to increase muscle mass *in vivo*. The following data demonstrate that administration of an ActRIIB-Fc fusion polypeptide increases muscle mass in an *in vivo* animal model of muscle atrophy. These experiments were conducted during April of 2007, with the analysis completed in June 2007.

Dexamethasone Model of Muscle Atrophy

4. To induce muscle wasting *in vivo*, we subcutaneously administered pellets releasing the glucocorticoid dexamethasone to 8 week old female C57Bl/6 mice with a time profile of 5 mg over 21 days. As a control, placebo pellets were administered. All animals were analyzed on day 14. Each cohort consisted of 6 mice. Data is presented as the cohort average with error bars representing the standard deviation; statistical significance was determined by student's t-test. Dexamethasone treatment reduced lean body mass in mice by approximately 15% (Attachment 1, bar 4) and skeletal muscle mass in the gastrocnemius muscle (by 31%) and quadriceps muscle (by 29%). See Attachment 2, bars 3 and 4. Furthermore, dexamethasone-treated mice had an approximately 37% reduction in muscle fiber cross sectional area. See Attachment 3, bar 4.

5. These results demonstrate that administration of dexamethasone *in vivo* results in a reduction in lean body mass, and induces severe atrophy in skeletal muscle, and therefore a dexamethasone treated mouse is an appropriate *in vivo* model of muscle disease/disorder.

ActRIIB-Fc Ameliorates Dexamethasone-Induced Loss of Lean Body Mass *In Vivo*

6. We next administered an ActRIIB-Fc fusion polypeptide to confirm that it would affect lean body mass in this *in vivo* muscle atrophy model. We prepared a human ActRIIB fusion protein comprising amino acids 23-138 of SEQ ID NO: 3 fused to the Fc region of a human IgG1 immunoglobulin (ActRIIB-Fc fusion polypeptide). Mice were dosed weekly by IP injection with a total weekly dose of 1 mg/kg, or 10 mg/kg, per animal for two weeks. As a control, mice were given a placebo pellet (as a dexamethasone control) and/or PBS (as an ActRIIB-Fc control). Attachment 1 shows the lean body mass, in grams. The pound symbol (#) indicates a statistically significant difference ($p<0.05$) when compared with the control mice.

7. As shown in Attachment 1, bars 2 and 3, when 1 mg/kg or 10 mg/kg of the ActRIIB-Fc fusion protein was administered to normal mice (i.e., mice that had not received dexamethasone), lean body muscle mass was increased by approximately 7% or 20%, respectively.

8. Administration of the ActRIIB-Fc fusion protein also increased the lean body mass of dexamethasone-treated mice. Dexamethasone treatment reduced lean body mass by 15%. Animals that received 1 mg/kg of the fusion polypeptide only

showed an 8% reduction in lean body mass, which was further improved to a 4% reduction in animals that received 10 mg/kg that was not significantly different from controls. See Attachment 1, bars 5 and 6.

9. These results demonstrate that administration of an ActRIIB-Fc fusion polypeptide to an animal suffering from glucocorticoid-induced muscle atrophy ameliorates the loss of lean body mass *in vivo.*

ActRIIb-Fc Ameliorates Dexamethasone-Induced Loss Of Skeletal Muscle *In Vivo*

10. After confirming that an ActRIIB-Fc fusion polypeptide can restore lean body mass, we tested the ActRIIB-Fc fusion polypeptide for its ability to ameliorate the loss of skeletal muscle mass in the same muscle atrophy model. The experimental procedures were the same as those described in paragraphs 4 and 6 above. At the end of the study, two different skeletal muscles were removed and weighed: the gastrocnemius and quadriceps muscles. Attachment 2 shows the mean tissue mass, in mg. The asterisk (*) indicates a statistically significant difference ($p<0.05$) when compared with control mice.

11. Administration of the ActRIIB-Fc fusion polypeptide restored muscle mass of both muscles in dexamethasone-treated mice: animals that received 1 mg/kg of the fusion polypeptide showed a 22% reduction in gastrocnemius muscle mass (compared to a 31% reduction in the control dexamethasone-treated mice that did not receive the ActRIIB-Fc fusion polypeptide) and a 23% reduction in quadriceps muscle mass (compared to a 29% reduction in control mice). See Attachment 2, bars 3-6.

Administration of 10 mg/kg of the fusion polypeptide was able to restore skeletal muscle mass to nearly normal (i.e. untreated) levels: the gastrocnemius muscle mass was reduced by 6%, and the quadriceps muscle mass was reduced by 8%. See Attachment 2, bars 7-8.

12. These results demonstrate that administration of an ActRIIB-Fc fusion polypeptide increases skeletal muscle mass in animals suffering from glucocorticoid-induced muscle atrophy.

ActRIIB-Fc Restores Fiber Cross-Sectional Area Following Muscle Atrophy

13. In a third set of experiments, the ActRIIB-Fc fusion polypeptide was tested for its ability to restore cross-sectional muscle fiber area in the same muscle atrophy model. The experimental details for the administration of dexamethasone and the ActRIIB-Fc fusion polypeptide were the same as those described in paragraphs 4 and 6 above. Attachment 3A shows a graphical representation of the mean muscle fiber cross sectional area (CSA), in square microns. CSA was measured from 200 fibers on each of 4 distinct histologic sections of the quadriceps for each mouse; each cohort had at least 5 mice. The asterisks (*) indicate a statistically significant difference ($p<0.05$) when compared with control mice by student's t-test. Attachment 3B shows photomicrographs of muscle fiber cross-sections.

14. Administration of 1 mg/kg or 10 mg/kg of the ActRIIB-Fc fusion protein to normal mice increased muscle fiber CSA by 9% and 28%, respectively. See Attachment 3A, bars 2 and 3.

15. Administration of dexamethasone reduced muscle fiber CSA by 37% reduction in muscle fiber CSA; however, administration of 1 mg/kg or 10 mg/kg of the ActRIIB-Fc fusion protein ameliorated this reduction to 27% and 7%, respectively. See Attachment 3A, bars 4-6. Representative muscle fiber cross sections are shown in Attachment 3B.

16. These results demonstrate that administration of an ActRIIB-Fc fusion polypeptide increases muscle in normal animals, and is able to restore or prevent the loss of individual muscle fiber that is associated with glucocorticoid-induced muscle atrophy *in vivo*.

17. In summary, administration of the ActRIIB-Fc fusion protein to mice with dexamethasone-induced muscle atrophy increased lean body mass, muscle mass (of both the gastrocnemius and the quadriceps muscle), and muscle fiber cross sectional area. These studies demonstrate that ActRIIB-Fc fusion polypeptide functions *in vivo* to increase muscle mass in a muscular disease/disorder.

18. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

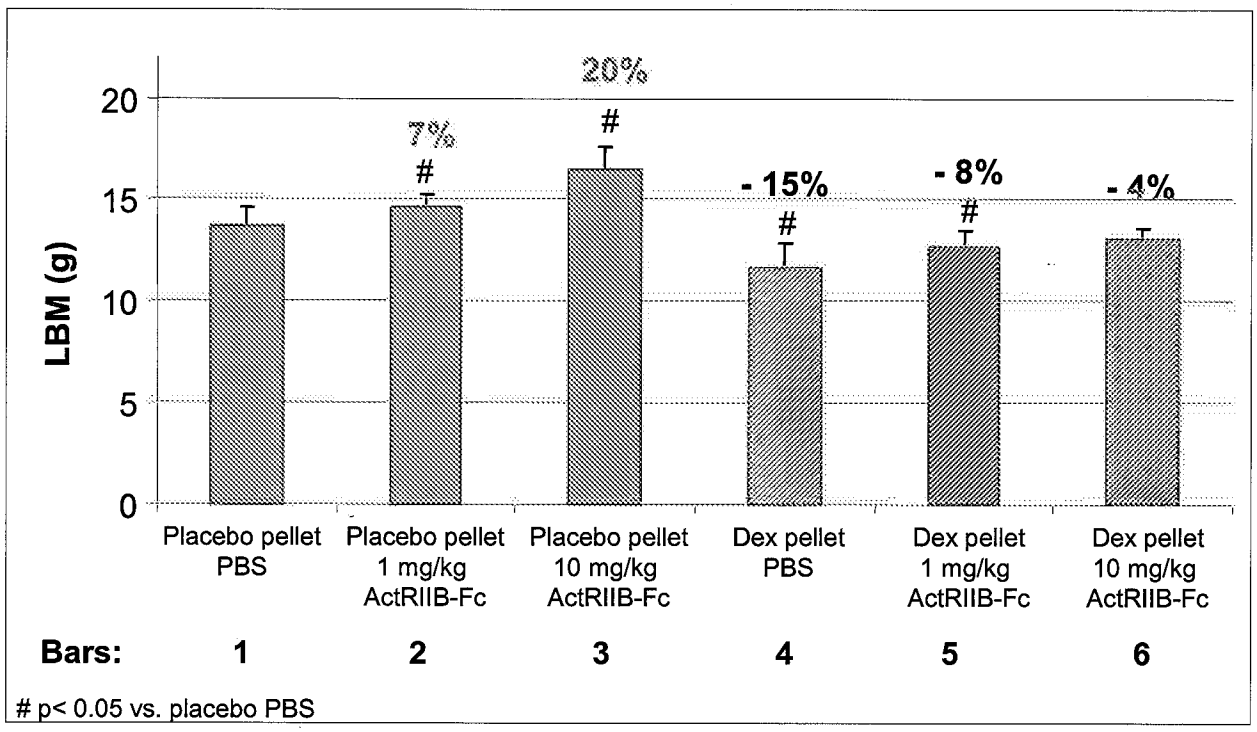
Dated: 8.28.07

By: _____

Paul Yaworsky, Ph.D.
Associate Director
Women's Health and Musculoskeletal Biology
Wyeth

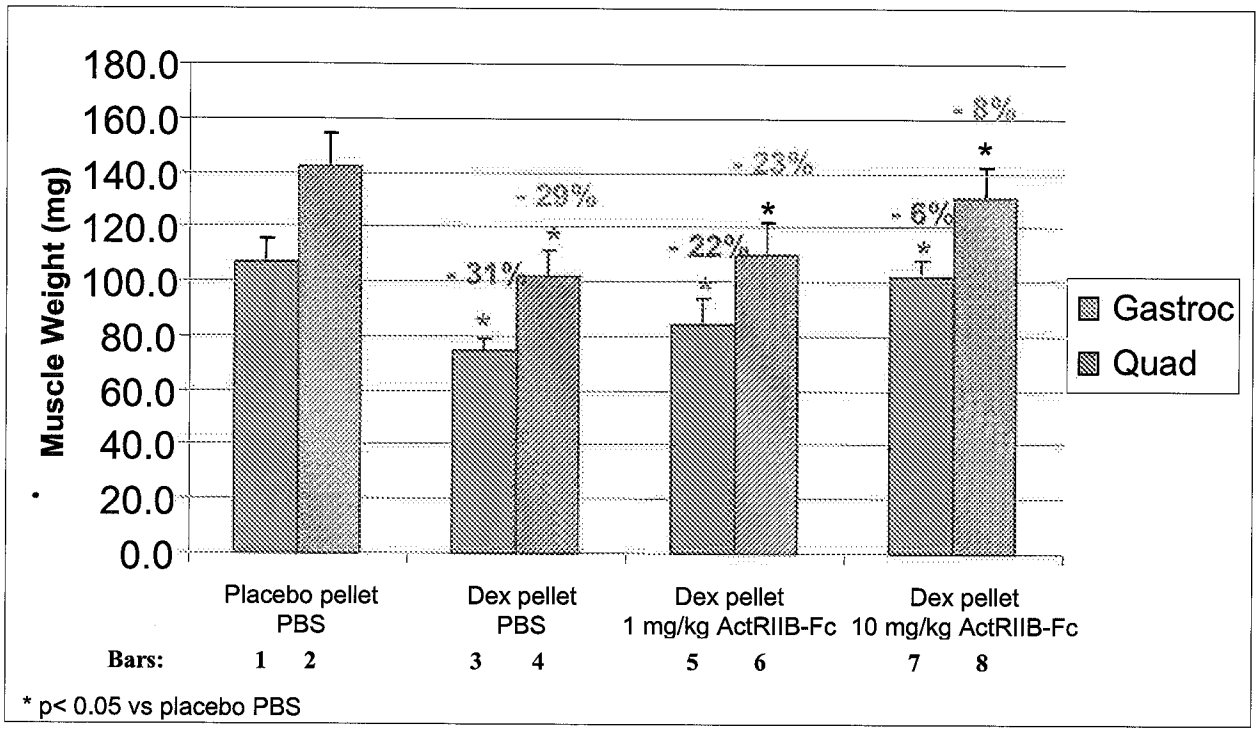
ATTACHMENT 1

ActRIIb-Fc Ameliorates Dexamethasone-Induced Loss of Lean Body Mass *In Vivo*



ATTACHMENT 2

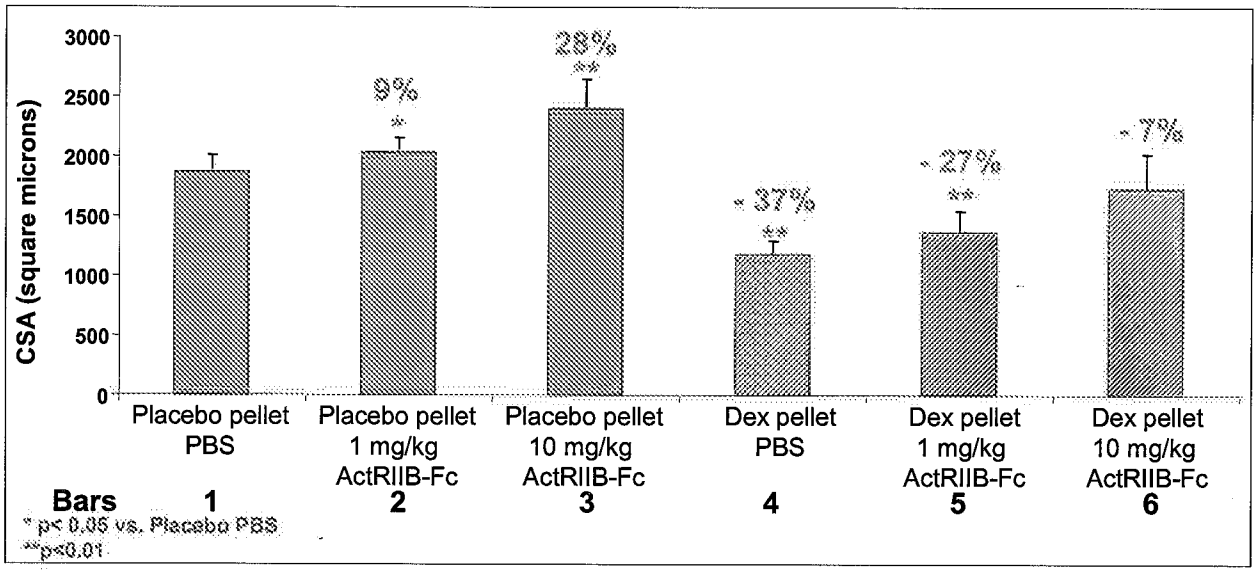
ActRIIB-Fc Ameliorates Dexamethasone-Induced Muscle Atrophy



ATTACHMENT 3

ActRIIb-Fc Restores Fiber Cross-Sectional Area Following Muscle Atrophy

A.



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